Evolution of microparasites and maintenance of parasite virulence: a review

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Abstract

Why do parasites harm their hosts? Initially, it has been thought that parasites should evolve to become avirulent because survival of the parasite is tightly linked to that of the host. However, for several infections it has been shown that some degree of harm is required for successful spread of the parasite. Subsequently, it has been suggested that if the rate of parasite transmission is correlated with virulence of the parasite, parasites need not evolve to avirulence. Using mathematical models based on this and other assumptions, many further predictions have been generated and a few of these have been tested experimentally. Along with the rapid growth of this adaptive theory of parasite evolution, several nonadaptive explanations for the existence of virulent parasites have also been proposed.

Keywords: evolution of virulence, conventional wisdom, adaptive theory, non-adaptive virulence, review, mathematical models

1 Introduction

Parasitism is an intimate association between organisms of two or more kinds, one in which, a parasite, obtains benefits from a host which it usually injures (Merriam-Webster dictionary). Although parasites are generally found as the result of degenerative evolution and might be considered as an inferior state of development, parasitism is widespread. While it is difficult to know how many parasites there are in Nature (in part because we do not know the all hosts), it has been estimated that 10-80% of species parasitize on other species to some extend (Windsor, 1998; Poulin and Morand, 2000).

While parasites are ubiquitous, we still do not have good understanding of why parasitism still exists. It has been (and to some extend still is) believed that parasitism is an inferior state and is simply due to a recent association between two species (Mims et al., 2001). According to this conventional wisdom, with time parasites should evolve to become avirulent. For several infections, this hypothesis has been rejected on the grounds that virulence is required for parasite transmission, and several alternative hypotheses have been formulated to explain the persistence of parasites.

In this paper, I review three hypotheses on the evolution of microparasites and maintenance of parasite virulence including the conventional wisdom (Section 3.1), adaptive theory (Section 3.2) and non-adaptive explanations (Section 3.3), with the main emphasis on the adaptive theory of parasite evolution. For these hypotheses I discuss their assumptions, predictions generated from the hypotheses, and their experimental tests. Importantly, in this review I focus only on the evolution of microparasites (or thereafter simply parasites) that are generally defined to include viruses, bacteria, protozoa (unicellular parasites), and fungi. The reader is also referred to several excellent reviews of this field emphasizing both experimental and theoretical results (Levin and Svanborg-Eden, 1990; Bull, 1994; Read, 1994; Frank, 1996; Ebert and Herre, 1996; Levin, 1996; Lipsitch and Moxon, 1997; Ebert, 1999; Read et al., 1999; Schall, 2002; Sabelis and Metz, 2002; Galvani, 2003; Ebert and Bull, 2003). But first, before going into details of these hypotheses, I will discuss what is virulence and what are the adequate measures of virulence.

2 What is virulence?

There have been many discussions on what virulence is in different fields (for example, see recent reviews by Poulin and Combes (1999) and Weiss (2002)). In microbiology, for example, virulence is defined as an ability of the parasite (mainly bacteria and viruses) to replicate in a host (Tortora et al., 1992; Mims et al., 2001). Genes providing the parasite with such an ability are therefore called "virulence factors" (Finlay and Falkow, 1997). In plant biology, parasite virulence is usually but not always associated with an ability of the parasite to infect the host (Read, 1994; Poulin and Combes, 1999; Thrall and Burdon, 2003). While it is hard to find a universally acceptable definition, in the field of the evolution of infectious diseases, virulence is generally defined as *the reduction in host's fitness due to infection with the parasite* (Bull, 1994; Ebert and Herre, 1996).

According to this definition, virulence of a parasite is proportional to the reduction in the number of offsprings, survived to reproductive age, produced by a parasite-infected host in comparison with an uninfected host. In practice, however, this parameter is rarely measured. Instead, other indirect alternative measures of the reduction of host fitness due to infection have been applied. Among the most widely used are the following: (i) the case mortality (a probability that an infected host will die following infection), (ii) the parasite-induced host mortality rate (the rate at which hosts in the population die due to infection with the parasite), (iii) the average life-span of infected hosts, (iv) the lethal dose 50, LD_{50} (an initial dose of the parasite required to kill 50% of infected hosts during the infection), and (v) the within-host parasite's growth rate r. While case mortality and host mortality rate are more direct measures of virulence, LD_{50} and r are clearly not.

Given many alternative measures to choose from, optimally, the virulence measure used should represent the reduction in host fitness due to the infection as close as possible. Sometimes, however, it is not achieved, and for some infections, conventionally used measures of virulence may be truly inadequate.

For example, many parasites that achieve high densities within their hosts cease host reproduction (Baudoin, 1975). If infection occurs early in life and is life-long, such castration represents a large reduction in host's fitness. On the other hand, since infected hosts generally do not die following infection and in some cases may survive longer because more resources are devoted to the growth (Baudoin, 1975; Ballabeni, 1995), such parasites are relatively avirulent if virulence is measured by the case mortality or host mortality rate (virulence measures employed in most mathematical models of parasite evolution).

In some cases, it might even be impossible to compare different infections by their virulence using alternative virulence measures. For example, which infection is more virulent for humans, smallpox or HIV? Smallpox can have case mortality up to 40% (Behbehani, 1983; Berche, 2001) while HIV is nearly 100% lethal (Buchbinder et al., 1994). On the other hand, the duration of infection with smallpox until the host's death or recovery is less than one month while in HIV infection it takes on average 10-12 years for infected hosts to die if untreated (Longini et al., 1989; Longini, 1990; Buchbinder et al., 1994; Mellors et al., 1996). This can be translated into the host mortality rate for smallpox $\alpha = 0.4/30 \approx 1.3 \cdot 10^{-2}$ day^{-1} and HIV $\alpha = 1/(10 \cdot 365) \approx 2.7 \cdot 10^{-4}$ day^{-1}. Obviously, two measures of virulence (case mortality and host mortality rate) rank these two infections differently. But which of these two measures is more appropriate to estimate virulence of each infection?

Since acute infections by definition are infections of short duration, it is likely that the parasite causing the infection will reduce host's reproductive success only if the host does not survive the infection. Therefore, if infection occurs early in life and does not impose any long-term consequences on subsequent survival and reproduction of hosts survived the infection, the most appropriate measure for virulence of parasites causing acute infections (such as smallpox) is the *case mortality*. In contrast, chronic infections often last for the life-span of an infected individual. If infected hosts reproduce less than uninfected hosts, then the *host mortality rate* due to infection, that is $\frac{\text{case mortality}}{\text{duration of infection}}$, is the appropriate measure for virulence of parasites causing chronic, persistent infections (such as HIV). Thus, smallpox and HIV cannot be compared by their virulence if one uses alternative measures of virulence. However, the infections can be compared if one estimates the true reduction of host fitness due to these infections, the task clearly more difficult than estimation of case mortality or host mortality rate.

3 Why are parasites virulent?

3.1 "You shall not murder": a conventional wisdom

> "Given enough time, a state of peaceful coexistence eventually becomes established between any host and any parasite... Throughout

> nature, infection without disease is the rule rather than the exception." (Dubos, 1965, p. 190)
>
> "It is a conflict between man and his parasites which, in a constant environment, would tend to result in a virtual equilibrium, a climax state, in which both species would survive indefinitely " (Burnet and White, 1972, p. 20-21)
>
> "In general terms where two organisms have developed a host-parasite relationship, the survival of the parasite species is best served, not by destruction of the host, but by the development of a balanced condition in which sufficient of the substance of the host is consumed to allow the parasite's growth and multiplication, but not sufficient to kill the host." (Burnet and White, 1972, p. 29)
>
> "...from an evolutionary point of view, successful microbes must avoid extinction, persist in the world, multiply, and leave descendants." (Mims et al., 2001, p. 3)

Parasites require their hosts for replication and transmission and death of the host often means death for the parasite. A conventional wisdom suggests that parasites should evolve to reduce the damage done to the host and eventually to become avirulent. This pacifistic view on parasite evolution became widely spread in part because of influential books by Dubos (1965) and Burnet & White (1972).

There are two major observations that are used in support of this hypothesis. First, many parasites do not cause severe disease in their natural hosts, i.e., in hosts where long coevolutionary history of the parasite and its host is known or suspected. For example, myxoma virus induces a very mild disease when infects its natural host, the American rabbit (Fenner and Ratcliffe, 1965). Simian Immunodeficiency virus (SIV) infection of natural hosts such as SIVsm infection of sooty mangabeys or SIVagm infection of green monkeys does not lead to immunodeficiency despite rapid replication of the virus and high rates of T-cell turnover (Rey-Cuille et al., 1998; Chakrabarti et al., 2000; Diop et al., 2000). Many Orthomyxo-, Arena-, and Hantaviruses cause asymptomatic infection in their natural hosts (influenza A in birds, arena and hantaviruses in rodents) (Murphy and Webster, 1996; Peters et al., 1996; Lednicky, 2003). Causing little pathology and being successfully transmitted are therefore ideal traits of the parasite.

Second, some parasites are extremely virulent when they encounter a novel host[1]. With time, parasite virulence (generally associated with the severity and incidence of the parasite-caused disease) declined. Several infections that have been brought into the New World by Europeans such as smallpox, measles, and influenza appear to follow this pattern. Initial severity of these infections in Indian populations is well known (Dubos, 1965; Burnet and White, 1972; Mims et al., 2001). Despite the fact that the reasons for such initial severity and its decline with time are still debated (Black, 1992), both these observations have been interpreted that parasites evolve to cause less harm to their hosts.

It is important to emphasize that the decrease in parasite virulence has not been directly measured in these examples. The only well documented change in virulence of a parasite after introduction into a new population is the evolution of myxoma virus in Australian populations of European rabbits (reviewed in (Fenner and Ratcliffe, 1965; Fenner and Fantini, 1999)). The initially introduced myxoma virus strain was very lethal to wild and laboratory rabbits (with case mortality $> 99\%$). In several years following the introduction, the average virulence level of the virus as measured in laboratory rabbits has declined (see Figure 1), in accord with the prediction of the conventional wisdom hypothesis. Later, however, rabbits became more resistant and that in turn led to the selection of more virulent strains of the virus that currently kills approximately 50% of wild and more than 99% of laboratory rabbits (Fenner and Fantini, 1999; Merchant et al., 2003b; Kerr et al., 2003; Merchant et al., 2003a, see Figure 1).

It is generally interpreted that conventional wisdom assumes that parasites should evolve to avirulence. While this might be generally correct, some examples on the evolution of infectious diseases given, for instance, by Dubos (1965) imply that host evolution may be an important factor and that changes in host resistance might be responsible for lowering virulence of human parasites. One example where most likely host evolution towards resistance resulted in a relative benign infection is again myxomatosis. The myxoma virus causes very mild disease in its natural host, American rabbits *Sylvilagus brasiliensis* and *S. bachmani*, and initially caused severe disease in European rabbits in Australia (Fenner and Ratcliffe, 1965; Fenner and Fantini, 1999). Although there have been changes in virulence of the virus since its first introduction in Australian rabbit populations as well as in the resistance of rabbits, the virus

[1] It should be emphasized, however, that these examples are rather exceptions than the rule since many such encounters most likely occur unnoticed due to inability of the parasite to replicate in a new host (Ebert, 1998).

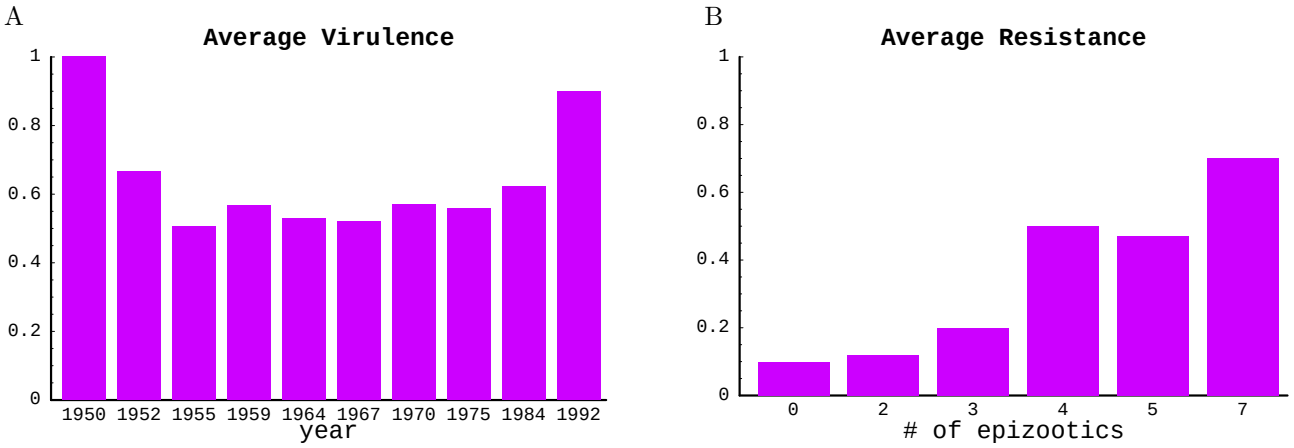

Figure 1: Changes in the level of average virulence of the myxoma virus and average resistance of rabbits after introduction of the virus in rabbit populations in Australia. Panel A shows the changes in average virulence. Average virulence was calculated using the prevalence data on the different strains of the virus and assigning arbitrary value 1 to the most virulent strain (grade I) and 1/5 to the least virulent strain (grade V). Data are taken from Fenner and Fantini (1999). Panel B shows the changes in average resistance after several epidemics at the lake Urana in Australia. Average resistance was calculated as a fraction of survived hosts after challenge of nonimmune rabbits with the virus of grade III virulence. Data are from Fenner (1983).

is still quite virulent. Immunological studies of myxoma pathogenesis in resistant and susceptible rabbits suggest that it is the host immune response that limits the spread of the virus in resistant rabbits, but causes pathology in susceptible hosts (Best and Kerr, 2000; Kerr and McFadden, 2002). Similarly, SIVsm/HIV-2 is non-pathogenic in its natural host, sooty mangabeys, yet causes AIDS in infected humans (Peeters et al., 1991; Rey-Cuille et al., 1998; Chakrabarti et al., 2000). Thus, host evolution rather than parasite evolution may be the reason why some infections are mild.

One of the shortcomings of the conventional wisdom hypothesis is that it is hard to test it. For some infections, however, this hypothesis has been rejected on the grounds that some virulence was required for the infection to be transmitted. For example, water diarrhea caused by *V. cholera* appears to enhance the transmission rate of the bacteria. Some other parasites are transmitted only from dead hosts and thus killing the host may be advantageous for such parasites (Lafferty, 1999; Ebert and Weisser, 1997; Ebert et al., 2000).

While some infections of humans, such as smallpox and TB, have been in the human population for thousands of years, it still could be argued that not enough time has passed to select for strains with reduced virulence. This argument of "insufficient time" that could be applied to many virulent infections is not easy to reject because we don't have good estimates of how long it should take for a parasite to become avirulent. From the serial passage experiments (SPEs) we know that parasites can evolve quite rapidly (reviewed in Ebert (1998)). However, it is not clear if rates of parasite evolution observed in SPEs are close to natural since in SPEs very high numbers of parasites are transferred in a non-natural way such as using syringe needles. On the other hand, a rapid change in average virulence of the myxoma virus after the initial introduction (Fenner and Ratcliffe, 1965) and a rapid decline in prevalence of toxin-producing bacteria (*C. diphtheriae* and *B. pertussis*) following introduction of anti-toxin vaccines (Schneerson et al., 1996; Taranger et al., 2001) does suggest that parasites may evolve their virulence in a matter of years.

This is at least one area in which theory could provide some answers. It is often emphasized that parasites may evolve more quickly than their hosts because of their large population sizes and short generation times (Mims et al., 2001). However, new infections are generally initiated by only a few parasites (Sacristan et al., 2003), and therefore, the effective population size of parasites may be much less than the size reached by the parasite in a given infected host. How this and other factors may affect the rate of parasite evolution to increased/decreased virulence has not yet been addressed (De Leo and Dobson, 2002).

3.2 "Enlightened theory": the adaptive theory of parasite evolution

The conventional wisdom has failed to realize that parasites evolve to maximize their reproductive success and not the duration of infection or the probability of species survival. In some cases reproductive success of a parasite can be calculated as the number of newly infected hosts during the infection. Although a large duration of infection is beneficial to the parasite, having a shorter infection may be still advantageous if shorter duration is compensated by an increase in the infection rate of new hosts. Therefore, this trade-off between the rate of infection and the duration of infection will determine the optimal parasite infectivity and duration of infection if parasites evolve in the presence of such a trade-off. A shorter duration of infection generally is associated with higher virulence of the parasite measured by the host mortality rate. The suggestion that parasites evolve in the presence of such a trade-off (or additional trade-offs) is the backbone of the adaptive theory of parasite evolution. There has been a number of experimental studies aimed to examine the assumptions of the adaptive theory and theoretical studies aimed to make further predictions on the evolution of parasites assuming that the trade-offs are present.

Anderson and May (1982) in their influential paper proposed a theoretical framework for the analysis of the evolution of parasites. For the epidemiological spread of the infection caused by a parasite, they considered the basic reproductive number of the parasite, R_0, that is, the average number of new infections caused by an infected host introduced into a wholly susceptible population. For directly transmitted infections R_0 is:

$$R_0 = \frac{\beta N}{d + \alpha + \nu}, \quad (1)$$

where β is the rate of parasite transmission from infected hosts[2] and α, d and ν are the rate constants for the parasite-induced and natural host mortality and recovery, respectively, and N is the density of susceptible hosts. In this model, the parasite-induced host mortality rate α is taken as a measure of virulence.

If an infected host can be occupied only by one parasite strain, then the parasite with the maximal R_0 will exclude others from the population (Anderson and May, 1982; Bremermann and Thieme, 1989). If β, α, and ν are all independent, then selection will favor parasites that are highly infectious ($\beta \to \infty$), avirulent ($\alpha \to 0$), and causing persistent infections with no recovery ($\nu \to 0$). Based on experimental observations Anderson and May proposed that these parameters at least for some infections may not be independent. For example, transmissibility and host recovery rate may depend on the parasite-induced host mortality rate, $\beta = \beta(\alpha)$ and $\nu = \nu(\alpha)$. Such dependencies are often called trade-offs even though the correlation $\beta = \beta(\alpha)$ when it exists is generally positive. For some appropriately chosen functions $\beta(\alpha)$ and $\nu(\alpha)$, the maximum of R_0 is achieved at intermediate levels of α.

Anderson and May used this theory to predict the optimal level of virulence of the myxoma virus evolving in populations of European rabbits in Australia after its initial introduction in 1950 (Figure 1). After the introduction of a very virulent virus strain, virus virulence declined dramatically for the following several years and was stably maintained for some time at intermediate levels. From laboratory experiments it became clear why virus strains with low and high virulence had lower fitness than strains with intermediate virulence: strains with low virulence caused only mild diseases in rabbits which survived for long periods of time. However, the probability of virus transmission from such rabbits was very small because of small densities of the virus in the skin lesions of infected hosts. Thus, even at long duration of infection, these strains do not obtain high total transmission. Similarly, highly virulent strains, were transmitted more efficiently but only for a very short time, obtaining low total transmission (Fenner and Ratcliffe, 1965).

Using the data on the case mortality ($M = \alpha/(\alpha+\nu)$) and the average duration of infection in hosts that died following infection ($\Delta \approx \alpha^{-1}$) caused by different viral strains (grades I-V), Anderson and May estimated the trade-off between the host recovery rate ν and the host mortality rate α (Anderson and May, 1982; May and Anderson, 1983, Figure 2). Using this trade-off and assuming that transmissibility β does not depend on virulence α, they found the optimal virulence level, at which R_0 is maximal (Figure 2). The obtained value, $\alpha^*_{theory} \approx 0.013\ day^{-1}$, was close to the observed $\alpha^*_{observ} \approx 0.041\ day^{-1}$ (Fenner and Ratcliffe, 1965; Anderson and May, 1982). This prediction was then improved by assuming a positive correlation between the probability of parasite transmission from infected to uninfected hosts for two vectors, fleas and mosquitoes (Massad, 1987; Dwyer et al., 1990, Figure 2). Thus, the trade-offs between parasite transmissibility, host recovery rate and parasite-induced host mortality rate determine the level at which parasite fitness is maximal.

Some of these trade-offs seem to be intuitively obvious. For example, higher average parasite load in an infected host may generally lead to higher transmissi-

[2] and simultaneously, the rate of infection of susceptible hosts.

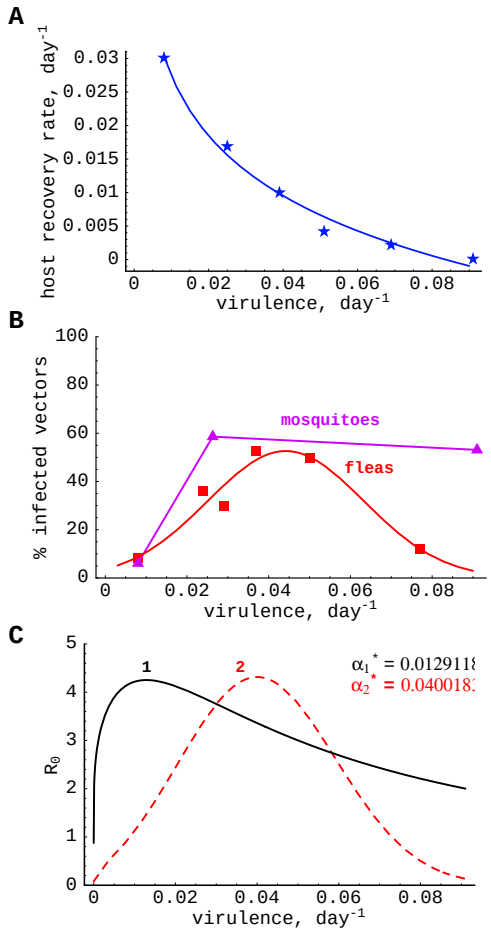

Figure 2: Measured trade-offs for the naturally occurring strains of the myxoma virus. Panel A shows the trade-off between the host recovery rate ν and parasite-induced host mortality rate (virulence) α. Panel B shows the trade-off between the probability of parasite transmission from an infected to susceptible host for two vectors, mosquitoes ($\triangle$) and fleas ($\square$), and virulence. Data are taken from Fenner et al. (1956), Mead-Briggs and Vaughan (1975), and Anderson and May (1982). Panel C shows the changes in the basic reproductive number R_0 calculated when only the trade-off between ν and α is known (1), or when both trade-offs are used (2). To calculate R_0 the following approximations for the trade-offs were used: $\nu = -0.032 - 0.013\log(\alpha)$ (Anderson and May, 1982), $\beta = \exp\left[-\frac{(\alpha-0.044)^2}{0.0007}\right]$ (fleas).

bility and higher virulence, thus leading to a positive correlation between the two traits. For some infections such correlation has indeed been found (see Table 1 for some examples) but not for others (Davies et al., 2001).

Two points need to be emphasized. First, many infection of practical interest have not been rigorously tested as to whether there are trade-offs that may constrain their evolution. Such studies should establish that (1) there is a variation in the parasite population in the degree of virulence, and (2) different parasites strains have different fitnesses (for example, total transmissions) upon which natural selection can act. Despite the absence of such knowledge, the trade-off hypothesis has been widely used to explain changes in virulence of many distinct parasites without actual demonstration that the trade-offs for such infections exist (Ewald, 1994; Dieckmann et al., 2002).

Second, in most of the studies where trade-offs have been established, the causes of these trade-offs are generally not well understood. It is likely that the causes of the trade-offs can be simple or complex for different host-parasite associations.

For example, the correlation between transmissibility and virulence may be simply because of the direct linkage between these two traits. For a horizontally transmitted microsporidian parasite *Glugoides intestinalis* of the water flea *Daphnia magna* the following relationships have been established: the number of parasite spores per host is inversely correlated with the life-span of infected hosts (i.e., the duration of infection) but is positively correlated with the amount of parasites expelled into the environment. A higher spore density in the environment, on the other hand, leads to a higher probability of infection of uninfected hosts (Ebert, 1994; Mangin et al., 1995; Ebert and Mangin, 1997). Thus, in this case, the correlation between virulence ([life-span of infected hosts]$^{-1}$) and transmissibility is simply mechanical: an increase in the parasite production rate may shorten the infection, but simultaneously leads to an increase in the probability of infection of a new host.

One the other hand, although measured, the underlying mechanisms of the correlation between transmissibility and virulence for the malaria parasite of rodents, *Plasmodium chabaudi*, are less clear because of the more complex nature of the infection (Mackinnon and Read, 1999). In this infection virulence, often measured by host morbidity, weight loss and case mortality following the infection, is caused by the replicating asexual stage of the parasite (merozoites) mainly by depletion of red blood cells. Transmission to the mosquito vector is due to a terminally differentiated sexual stage (game-

infection	transmission	virulence measure	references
myxoma virus in rabbits	mosquitoes, fleas	case mortality	(Fenner et al., 1956; Mead-Briggs and Vaughan, 1975)
influenza A in mice	direct (airborne)	pathology of lungs	(Schulman, 1967, 1970)
malaria (*P. chabaudi*) in mice	mosquitoes/needle	anemia/body wasting	(Mackinnon and Read, 1999)
G. intestinalis in Daphnia	fecal-oral	host survival time	(Ebert, 1994; Ebert and Mangin, 1997)
phage f1 in *E.coli*	environment	host generation time	(Messenger et al., 1999)
CM virus in tomato plants	direct/vector-borne	general pathology	(Escrui et al., 2003)

Table 1: Examples of infections for which the correlation between the transmission success of a parasite and parasite virulence has been established.

tocytes). Because of the complexity of the differentiation pathway of the asexual to sexual stage in *Plasmodium sp.* (Taylor and Read, 1997; Dyer and Day, 2000), the underlying mechanisms of the correlation between transmissibility and virulence are not well understood.

Importantly, for parasite strains recovered from host populations in Nature, a correlation between transmissibility and virulence may be observed even without a physical linkage between these two traits. Such a correlation may simply arise because more virulent parasite strains are evolutionary selected for higher transmissibility. For example, theoretical studies on the evolution of parasites in spatially heterogeneous environments suggest that for a given level of virulence (a probability of host dying following infection) parasites with too low and too high rates of transmission will not be maintained in the host population (Haraguchi and Sasaki, 2000; Rauch et al., 2003). This is because parasites with too low transmissibility on average kill their hosts before they are transmitted while parasites with high transmissibility infect and kill locally all susceptible hosts and are not able to be transmitted to next cluster of hosts (Haraguchi and Sasaki, 2000; Rauch et al., 2003).

Despite these shortcomings, the adaptive theory forms the basis for the analysis of the evolution of infectious diseases. Its great advantage is that it relies on the trade-offs to predict evolution of parasites, and therefore, can be rejected if the trade-offs for the parasite-induced disease are not observed.

Further specific predictions of the adaptive theory, their tests and critique

Since Anderson and May, many theoretical studies have made specific predictions on how parasites should evolve in different conditions (for a relatively recent theoretical review see Frank (1996)). However, only a few of such predictions have been tested experimentally (see, for example, an excellent discussion by Schall (2002) for the testing of different predictions of the adaptive theory applied to malaria parasites of lizards).

3.2.1 Natural host mortality rate

Many models of parasite evolution predict that an increase in the parasite-independent host mortality rate should lead to selection of parasites that kill their hosts more rapidly and therefore are more virulent (Frank, 1996; Ebert and Mangin, 1997; Day, 2002b). This is simply because a shorter life-span of the host often leads to a shorter duration of infection that in turn selects for more rapidly replicating parasites. This can also be seen by maximizing the basic reproductive number R_0 given in eqn. (1) with respect to α assuming a positive correlation $\beta = \beta(\alpha)$. The optimal virulence level obtained at $\beta = \beta_0\alpha/(\alpha + c)$ and given by eqn. (4) increases with increasing natural host mortality rate d. Similarly, a longer host life-span would lead to selection of slower replicating parasites with lower virulence. For several experimental systems these predictions have been tested with variable success (Ebert and Mangin, 1997; Ebert, 1998; Elena, 2001; Cooper et al., 2002).

In serial passage experiments (SPEs) where parasites are manually transmitted from an infected to a new host, parasites evolve higher within-host growth rate and concurrently virulence if the transmission event occurred early in infection (reviewed in Ebert (1998)). Since early transmission mimics high host mortality rate, this observation is consistent with the prediction of the adaptive theory. Similarly, parasites that are serially passaged late during the infection evolve lower growth rate and virulence (Dobson and Owen, 1977; Elena, 2001; Cooper et al., 2002).

While consistent with the theory, these results cannot be directly applied since transmission in SPEs is generally done manually at one fixed time point and therefore does not correspond to the natural way of parasite transmission. Ebert and Mangin (1997) attempted to test the above prediction using a quasi-natural setting. They allowed a microsporidian parasite (*Glugoides intestinalis*) to evolve in populations of *Daphnia magna* while applying two regimes with high and low natural host mortality. High natural host mortality was achieved by transferring only 10-20% of hosts into a new aquarium, while the control (low host mortality) was left unma-

nipulated. Surprisingly, parasites in the high mortality rate regime evolved lower growth rate and virulence (measured as the life-span of infected hosts) than those from the low mortality regime. The authors speculated that in unmanipulated populations a longer exposure to the parasites in the water led to an increased frequency of hosts infected with several distinct strains of the parasite (i.e., multiply infected hosts). This in turn selected for more rapidly growing parasite strains (Ebert and Mangin, 1997, Section 3.2.4). Although direct evidence for the occurrence of multiple infections in this experiment was lacking, theoretical analysis does suggest that when multiple infections are allowed, higher virulence is expected to evolve at low natural host mortality rate (Gandon et al., 2001a). This is simply because the longer duration of infection increases the probability of an already infected host to be super-infected with a more virulent parasite strain forcing parasites to become more virulent.

Other particular "details" of the infection may also change the prediction of how natural host mortality affects the optimal level of parasite virulence. For example, if the duration of infection is much shorter than the average host life-span, then reduction in the host life-span should not dramatically affect the optimal level of parasite virulence. Furthermore, theory suggests that higher host mortality can select for lower virulence depending on whether there is an interaction between parasite-induced and natural host mortality rates and on how virulence is measured (Williams and Day, 2001; Day, 2002b; Choo et al., 2003). These examples clearly illustrate that particular details of a given parasite-host association are important in predicting the evolution and the optimal level of parasite virulence even if the basic assumptions of the adaptive theory (such as the trade-offs outlined above) are fulfilled.

3.2.2 Host recovery rate/host resistance

It seems to be difficult to make general predictions on how host resistance would affect the evolution of parasites because of many ways the resistance can be provided. In plants and invertebrates resistance is often defined as inability of the parasite to infect the host (Thrall and Burdon, 2003; Rolff and Siva-Jothy, 2003). In contrast, in vertebrates, host resistance is often associated with the host ability to mount an effective immune response to quickly clear the infection.

General theory predicts that an increase in the recovery rate should select for more virulent parasites (Frank, 1996; Antia and Lipsitch, 1997; van Baalen, 1998; Gilchrist and Sasaki, 2002; Day and Burns, 2003; Andre et al., 2003). This is, similar to the previous case, because a higher recovery rate leads to a shorter duration of infection forcing parasites to evolve higher growth rate and virulence (see also eqn.(4)).

In contrast, increased host resistance to the infection (which can be achieved by both higher recovery rate and resistance to initial infection) may select for higher or lower virulence level depending on particular mechanisms of resistance (Gandon and Michalakis, 2000). As far as I know there have been no studies where these predictions have been rigorously tested. However, an increase in virulence of the myxoma virus following an increase in resistance of rabbits to the infection in the past few decades in Australia is consistent with the theoretical prediction (Fenner and Fantini, 1999, Figure 1).

Gandon et al. (2001b) have proposed that imperfect vaccines that, on the one hand, increase host resistance, but, on the other hand, allow replication and transmission of parasites, may lead to evolution of parasites with lower or higher virulence depending on the type of the vaccine. Vaccines blocking new infections or transmission from infected hosts are predicted to select for parasites with decreased virulence because they would reduce the intensity of intra-host competition between unrelated parasite strains resulting from superinfection (Section 3.2.4). In contrast, vaccines that reduce the within-host replication rate of parasites or their virulence are expected to select for parasites with increased virulence, because such vaccines remove the cost of virulence (Gandon et al., 2001b, 2003).

The assumptions of the mathematical model and generality of its predictions have been heavily criticized (Smith, 2002; Ebert and Bull, 2003; Andre et al., 2003), in part because the theoretical prediction that vaccination against toxins (i.e., virulence) should select for parasites with increased virulence was in contrast with the observed reduction in prevalence of toxin-producing *Corynebacterium diphtheriae* and *Bordetella pertussis* after introduction of anti-toxin vaccines (Soubeyrand and Plotkin, 2002). Toxin production bears a cost and in the presence of anti-toxin immunity, bacteria not producing toxins have selective advantage. Although *ad hoc* changes in the model to include the toxin cost led to "improved" predictions, this example again emphasizes the role of particular details in predicting the evolution of parasites.

3.2.3 Epidemic vs. endemic diseases (early vs. late transmission)

The adaptive theory assumes that parasites evolve to maximize their reproductive success. From theoretical

studies it became clear that for epidemic and endemic infections the reproductive success of parasites might be calculated in different ways (Frank, 1996). For epidemic infections the number of susceptible hosts is large and parasite strains that infect the hosts more rapidly will have selective advantage (Knolle, 1989; Lenski and May, 1994). Thus, in epidemic infections parasites will maximize the net growth rate of the number of infected hosts, that for directly transmitted diseases in the absence of the within-host competition is simply:

$$r = \beta N - (\alpha + d + \nu), \quad (2)$$

where the parameters are the same as in eq. (1). In contrast, for endemic diseases there is always dearth of susceptible hosts, so parasites that infect the maximum number of hosts during the infection, will have selective advantage. Thus, in endemic infections at some conditions[3], parasites will maximize their basic reproductive number R_0 that for directly transmitted diseases is given by eq. (1) (Anderson and May, 1982; Bremermann and Thieme, 1989; Frank, 1996).

If there are (appropriate) trade-offs between parasite characteristics both fitness measures are maximized at intermediate values of virulence. For example, if $\beta = \beta_0\alpha/(\alpha + c)$ and ν = const, the optimal virulence for two infection types are:

$$\alpha^*_{epidem} = \sqrt{cN\beta_0} - c, \quad (3)$$
$$\alpha^*_{endem} = \sqrt{c(d+\nu)}. \quad (4)$$

where $\alpha^*_{epidem} > \alpha^*_{endem}$ for any parameter combination. It can be also shown (see Appendix) that $\alpha^*_{epidem} > \alpha^*_{endem}$ for any trade-off $\beta = \beta(\alpha)$ if the host recovery rate depends weakly on virulence. Importantly, using eqns. (3)–(4) it is clear that an increase of the host population size N and/or transmission rate constant β_0 (proportional to the contract rate) may dramatically increase optimal virulence of epidemic infections but not of endemic infections (Frank, 1996).

As far as I know there have been no studies designed to test this prediction of the adaptive theory. There are, however, several observations that are consistent with it. First includes the increase in virulence of parasites in SPEs (Ebert, 1998). In these experiments, parasites are generally transmitted early in the infection thus mimicking the initial phase of an epidemic. On the other hand, selection for later transmission in SPEs that mimics an endemic leads to reduced virulence (Dobson and Owen, 1977; Elena, 2001; Cooper et al., 2002). For a more general discussion on how timing of transmission may affect the optimal level of parasite virulence, see (Day, 2003).

[3]These exclude the presence of within-host competition and density-dependent effects in host reproduction and infection (Nowak and May, 1994; May and Nowak, 1995; Bonhoeffer and Nowak, 1994; Dieckmann, 2002).

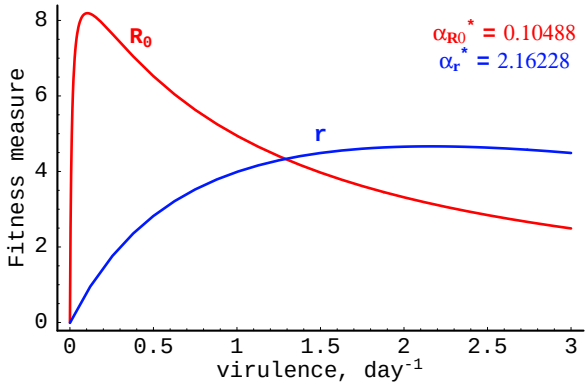

Figure 3: The relationship between the basic reproductive number R_0, the net growth rate of the epidemic r and the parasite-induced host mortality rate α. The correlation between the transmission rate and virulence is assumed in the form $\beta = \beta_0\alpha/(\alpha+c)$. Parameters are: $\beta_0 = 0.01$, $d = 0.001$, $c = 1$, $\nu = 0.01$, $N = 10^3$. Optimum levels of virulence for epidemic and endemic diseases are: $\alpha^*_{epidem} \approx 2.16$ and $\alpha^*_{endem} \approx 0.11$. As shown in the Appendix if ν = const then for any trade-off $\beta = \beta(\alpha)$, $\alpha^*_{epidem} > \alpha^*_{endem}$.

Second, it has been widely argued by Paul Ewald that higher host densities and high rates of parasite transmission are responsible for outbreaks of highly virulent parasites including influenza, cholera and HIV (Ewald, 1991, 1994). Although these arguments are consistent with the theoretical analysis, experimental data used in support of this prediction may have other interpretations (Frank, 1996). In addition, there are additional reasons to question the generality of these predictions applied to these particular infections as the absence of clear trade-offs between virulence and other traits (Ebert and Bull, 2003).

HIV in particular has drawn much attention in part because of its extreme lethality. For example, for HIV in particular and for sexually transmitted diseases (STDs) in general, it has been suggested that an increased contact rate may select for lower virulence because of the law of diminishing returns (Lipsitch et al., 1995). STDs should also be less virulent if they reduce sexual activity of the host (Knell, 1999). Why HIV is virulent still remains an open question. Ewald suggested that HIV evolved from a less virulent form because of increased contact rate (Ewald, 1991). Obviously, this need not be the case, because rhesus macaques infected with SIVsm/HIV-2, a natural parasite of sooty mangabeys,

progress rapidly to AIDS and die (Staprans et al., 1999; Buckley et al., 2003).

We can however ask a question of whether HIV will evolve to a more/less virulent form (Ganusov, 2003). Levin and Bull (1994) suggested that host immunodeficiency due to HIV infection may result from the within-host, short-sighted evolution of the virus and may have nothing to do with the rate of virus transmission (see Section 3.3). Therefore, according to this hypothesis changes in opportunities for transmission should not affect the optimal virulence of HIV.

However, it has been shown that the duration of HIV infection is inversely correlated with the virus density in plasma of infected hosts early in the asymptomatic period of the infection called the set-point (Mellors et al., 1996; Arnaout et al., 1999; Staprans et al., 1999; Goto et al., 2002). Similarly, viral load is positively correlated with the probability of heteterosexual transmission of HIV (Pedraza et al., 1999; Quinn et al., 2000; Gray et al., 2001). Such relationships indicate that there might be a positive correlation between HIV transmissibility and virulence (measured as the inverse of the duration of infection) for viral strains with different set-points. Although both host and virus factors play an important role in determining the rate of progression to AIDS (Deacon et al., 1995; Magierowska et al., 1999; Staprans et al., 1999; Buckley et al., 2003; Campbell et al., 2003; Theodorou et al., 2003; Trkola et al., 2003), their relative contribution to the disease progression is not yet understood (Theodorou et al., 2003). Formally, the correlation between transmissibility and virulence for HIV strains can be established if genetically identical hosts are infected with different strains of the virus. Given the lack of inbred strains of monkeys, this can be done, for example, by observing the disease progression in identical twins infected with different viral strains (Segal and Hill, 2003).

If the correlation between HIV transmissibility and the duration of infection is established, the adaptive theory suggests that HIV virulence will evolve. The exact value of the optimal level of HIV virulence will be then determined by (1) the exact shape of the correlation, (2) whether it is endemic or epidemic, and (3) what exactly is maximized by the virus (Ganusov, 2003).

For example, if the majority of the virus transmission occurs *only* during the acute infection, then we cannot predict HIV evolution since currently there is no relationship between viral load in acute infection and the rate of progression to AIDS (Staprans et al., 1999). Theoretical analysis suggests that during initial stages of the HIV epidemic the majority of the virus is transmitted early during the infection (Levin et al., 1996, 2001) but the relative contribution of acute infection and asymptomatic phase in such early transmission is not known. If, however, all transmission occurs within the first year of the infection given that the acute phase lasts for approximately one month, higher viral loads (and therefore faster progression to AIDS) in the asymptomatic phase may be advantageous to the virus during an epidemic. Selection (if any) may change as the infection reaches the endemic regime. Thus, predictions on whether an infection becomes more or less virulent depends critically on whether it is epidemic or endemic even if the trade-offs for the infection are established.

3.2.4 Within-host competition: mutation, co- and super-infection

Some of the predictions of the adaptive theory given in previous sections are based on the assumption that only one parasite strain can occupy a given host. In many instances this needs not be true. For example, *Daphnia magna* can be repeatedly infected with the same microsporidian parasite (Ebert, 1995). Humans infected with malaria often harbor several different strains of the parasite (Read et al., 2002). There have been several theoretical results suggesting that allowing competition between different parasite stains within one host, will select for increased virulence. Parasites evolve higher virulence in this case because of the risk to share the host with a more virulent parasite strain[4]. The increase in the number of parasite strains occupying the same host may result from mutation (Bonhoeffer and Nowak, 1994) and co- or superinfection (Sasaki and Iwasa, 1991; Frank, 1992; Nowak and May, 1994; May and Nowak, 1995; van Baalen and Sabelis, 1995; Frank, 1996; Mosquera and Adler, 1998; Leung and Forbes, 1998). Importantly, many predictions of the adaptive theory change if the within-host competition between parasite strains in multiply infected hosts is allowed. For example, higher natural host mortality rate would lead to selection of parasites with lower virulence. This is because with shorter infection there is less chance of multiple infections, and in particular superinfections, to occur (Gandon et al., 2001a).

The prediction of the adaptive theory that, in the presence of within-host competition, parasites should evolve to higher virulence, has been tested in several systems. As described in Section 3.2.1, it was hypothesized that multiple infections were responsible for in-

[4] In some cases, however, cooperation between different parasite strains may increase the efficacy of host exploration (Turner and Chao, 1999); such parasites are expected to evolve lower virulence (Brown et al., 2002).

crease in virulence of a microsporidian parasite *G. intestinalis* evolved in a population of *Daphnia magna* with low natural host mortality rate (Ebert and Mangin, 1997). Mixed-clone infections of mice with malaria parasite *P. chabaudi* result in higher maximum weight loss of the host (Taylor and Read, 1998; Read et al., 2002) that correlates well with other measures of virulence in this experimental system (Mackinnon and Read, 1999). In another experimental system, virulence of parasites infecting fig wasps is determined by how many different wasps (generally infected with distinct parasites) pollinate a given fig. If a fig is pollinated by several wasps, parasites infecting such wasps are on average more virulent than parasites infecting wasps that pollinate only unpollinated figs (Herre, 1993). Higher virulence of such parasites is most likely due to an increased level of competition with unrelated parasites within the same fig (Herre, 1993; Frank, 1996). Finally, the assumption that multiple infections are more virulent was necessary to correctly predict the epidemic of the *Cucumber mosaic virus* in the population of tomato plants (Escrui et al., 2003).

On the other hand, some observations of malaria infection of humans or trypanosome infection of bumblebees suggest that single infections may be as virulent as mixed infections (Imhoof and Schmid-Hempel, 1998; Read et al., 2002).

Clearly how multiple infections occur and the type of infection is important in determining whether and how multiple infections will affect the parasite evolution. For example, during an acute infection, the probability of superinfecting an already infected host is very low because of the short duration of infection. Similarly, because of the short duration of infection, mutations are not likely to generate high diversity in the parasite population during the infection unless the mutation rate is extremely high. Therefore, the presence of different parasite strains in the initial inoculum is the most likely mechanism by which multiple infections may occur in acute infections. In contrast, during chronic infections all the three mechanisms (co-, superinfection and mutation) may lead to increased parasite diversity in infected hosts.

Importantly, for some medically important infections (such as *Plasmodium falciparum* infection of humans), the role of multiple infections on the severity of the disease is not well understood (Smith, 2002; Read et al., 2002). Despite this fact, there have been many theoretical models assuming that multiple infections are the main force in driving the parasite evolution (see, for example, Gandon et al. (2001b)). Clearly, more experiments with particular infections are needed to establish how multiple infections affect the within-host dynamics of different parasite strains, severity of the infection and transmission success of parasites.

3.2.5 Host heterogeneity

Many simple predictions of the adaptive theory are based on the assumption that parasites evolve in populations of identical hosts. Clearly this is not the case for any natural population where genotypic, phenotypic and age differences between different hosts exist. Little work has been done to understand how parasites evolve in such heterogeneous host populations.

Omitting many details of how heterogeneity can be generated and maintained (for a review see (Ebert and Hamilton, 1996; Ebert, 1999; Galvani, 2003)), it is generally believed that higher levels of host heterogeneity would select for less virulent parasites (Ebert, 1998, 1999). To reach this conclusion it is implicitly assumed that (1) parasites are not able to adapt to different host types simultaneously[5], and (2) adaptation to one host type such as to increase replication/virulence is traded-off with replication in other host types. Some experimental observations are consistent with the assumptions and the prediction (Ebert and Hamilton, 1996; Ebert, 1998).

For example, spread of infections in host populations with low genetic diversity (such as some human populations or agrocultures) often results in high host moralities (Black, 1992; Ebert, 1999). Similarly, many parasites when serially passaged in new genetically identical hosts (or in hosts with low genetic diversity), evolve to increase their virulence (Ebert, 1998). In accord with this increase, virulence generally decreases when it is measured in the original host (Ebert, 1998).

Importantly, many of these and other observations can be explained without assuming that host heterogeneity selects for parasites with low virulence. For example, high host mortalities during an epidemic are expected if there is a correlation between the rate of parasite transmission and virulence (Section 3.2.3). An increase in virulence of serially passaged parasites may be simply due to strong selection for more rapid growth and not due to low genetic diversity of hosts (Ebert, 1998). The discrimination between the last two explanations can be done in SPEs if hosts of different genetic backgrounds are being used. If increase in virulence during SPEs is due to low host diversity, changing hosts at random or at each passage should prevent parasites from adapting to one host type and virulence from esca-

[5]By host types I mean different host strains for single-host parasites or different host strains/species for multi-host parasites.

lating. If the increase in virulence is due to relaxing the requirement for transmission, virulence should increase despite the hosts being changed.

In one experiment, the latter prediction has been confirmed. Turner and Elena (2000) passaged vesicular stomatitis virus, originally growing in BHK cells, in two novel host cell types (HeLa and MDCK cells). There was an increase in the instantaneous growth rate of the virus when measured in the new cell type and its simultaneous reduction when measured in the original cell type or another cell type. However, when cell types used for passage where changed (at random or after each passage), the evolved strain increased its growth rate in *both* novel cell types but had a reduced growth rate in the original cell type. In another experiment, a strain of *P. chabaudi* that had been passaged in C57Bl/6J mice, increased its virulence in C57Bl/6J mice, but was also more virulent in two unrelated mouse strains, CBA/Ca and DBA/2, when compared to the unpassaged parasite strain (Mackinnon et al., 2002).

Given these contradictory results, there is still no good understanding of how host heterogeneity affects the optimal level of parasite virulence. As far as I know only one theoretical study formally has addressed this question. Ganusov et al. (2002) assumed that parasites causing acute infections in vertebrates evolve in the population of hosts that stochastically differ in their susceptibility to infection or their quality of the immune response. They found that in the absence of heterogeneity parasites evolve to an intermediate growth rate but kill no host. The latter is due to the fact that there is a high loss in total transmission when the parasite kills the host. When the level of host heterogeneity increases, the optimal level of parasite virulence measured as the case mortality increases as well. This is because in order to obtain the maximum total transmission, the parasite has to compromise between killing "susceptible" hosts and obtaining high transmission from "resistant" hosts (Ganusov et al., 2002). Thus, the analysis suggests that higher levels of stochastic heterogeneity should select for higher optimal level of parasite virulence.

In a recent study it has been proposed that virulence of *Neisseria meningitidis*, infecting an immunologically diverse host population, may be the result of selection for parasites with high mutation rates (Ancel Meyers et al., 2003). A high mutation rate increases the changes of the parasite to infect heterogeneous hosts but simultaneously increases the probability of killing an infected host by evolving highly virulent strains within the host. If this explanation of *N. meningitidis* virulence is correct, it is expected that the parasite, infecting a homogeneous host population, should evolve low mutation rate and consequently low (theoretically zero) virulence.

In contrast, Regoes et al. (2000) have found that when two hosts types are present in the population, parasites evolve lower virulence than when only one host type is present. This is because the authors assumed an explicit trade-of between parasite virulence in two host types. While parasites infecting only one host type may evolve infinite virulence, this explicit trade-off does not allow virulence to escalate when two host types are present (Regoes et al., 2000). This study, however, did not investigate how the *degree* of host heterogeneity affects the optimal level of parasite virulence.

At this point we need more theoretical and experimental studies to answer the question of how host heterogeneity affects the parasite evolution. The term *heterogeneity* may also be interpreted differently whether one considers several strains of one host species or different host species. This becomes particularly important since many parasites infect more than one host species (Woolhouse et al., 2001). We expect however that the answer may be different for different parasite-host associations.

3.2.6 Route of transmission

Different parasites have different mechanisms of spreading from infected to susceptible hosts including horizontal (direct, vector-borne, and fecal-oral) and vertical transmission (Anderson and May, 1991; Mims et al., 2001). There has been a great debate pioneered by Paul Ewald on whether the route of parasite transmission may be the most important factor in determining virulence of parasites (Ewald, 1983, 1988, 1991, 1994). The main theme of all these predictions is that increasing opportunities for transmission should select for parasites with high virulence and similarly, reducing opportunities for transmission should select for parasites with lower virulence.

For example, according to this hypothesis, since host mobility is not required and may be even deleterious for the transmission of vector-borne parasites, such parasites should on average be more virulent than parasites that are transmitted directly and that require host mobility for transmission (Ewald, 1983). Similar arguments are applied to waterborne infections causing diarrhoea because they can spread from immobilized hosts (Ewald, 1991; Ewald et al., 1998). Although comparative data on different parasites support this idea, other data, for example, on transmission rates of malaria parasites of lizards that differ in their virulence, do not (Schall, 2002). In addition, such comparative analysis across different parasite species without consideration of other details of infection has other shortcomings (Ebert

and Bull, 2003). Theoretical analysis also suggests that whether vector-borne parasites are more virulent than directly transmitted parasites depends critically on the morbidity costs and the time schedule of the parasite transmission and needs not be true in general (Day, 2001, 2002a). On the other hand, assuming a positive correlation between parasite transmissibility and virulence for directly transmitted and waterborne parasites, Ewald and De Leo (2002) have found that parasites that can be transmitted directly through contact and indirectly through environment (such as waterborne parasites) evolve higher virulence than parasites transmitted exclusively directly. In their model, the basic reproductive number of the parasite is composed of two R_0 that are related to each transmission mode and are dependent on parasite virulence α:

$$R_0(\alpha) = R_0^{dir}(\alpha) + R_0^{indir}(\alpha) = \frac{\beta(\alpha)N}{\alpha+d+\nu} + \frac{\beta_w(\alpha)\rho/mN}{\alpha+d+\nu}, \quad (5)$$

where β_w is the infection rate of susceptible hosts by parasites in the environment, ρ is the shedding rate of parasites into the environment by infected hosts, and m is the decay rate of the parasite in the environment (i.e., $1/m$ is the parasite longevity in the environment), and other parameters are the same as in eqn. (1).

The maximum of the total R_0 can be achieved at virulence levels that are higher or lower than the optimal virulence of parasites transmitted only directly (i.e., when $R_0^{indir} = 0$). The optimal level of virulence depends critically on the relative contribution of two transmission routes and *the trade-offs* imposed for two routes. The authors assumed a bell-shaped trade-off for the direct transmission ($\beta = \beta_1\alpha/(c_1 + \alpha^2)$) and linear or saturating trade-off for the indirect transmission ($\beta_w = \beta_2\alpha$ or $\beta_w = \beta_2\alpha/(c_w+\alpha)$). The verbal argument for choosing these functions is that in directly transmitted infections, higher virulence level while causing initial increase in the transmission rate, will eventually reduce the transmission rate due to host immobilization. Since indirect (waterborne) transmission is not affected by the immobilization, there is no decrease in transmission rate with increasing virulence (Ewald and De Leo, 2002). Unfortunately, to my knowledge there is no experimental data that demonstrate such functional forms of the trade-offs for different routes of transmission for cholera. Experimental tests would have to involve measurement of costs of morbidity for direct transmission and virulence-transmission trade-offs for both routes of transmission.

Nevertheless, at these "appropriately" chosen functions the authors indeed found that parasites that are transmitted both directly and indirectly evolve higher virulence than parasites transmitted exclusively directly. However, using similar trade-offs but changing constants describing the trade-off for indirectly transmitted parasites, I find that optimal virulence may be higher or lower than that for exclusively directly transmitted parasites, depending on the parameter values (Figure 4). This example demonstrates the major weakness of the "epidemiological" approach for understanding the evolution of parasites: in many cases not only the particular biological details of the modeled system are important, but the conclusions can be affected by the exact shape of trade-offs used in the analysis.

Other studies nevertheless suggest that if parasites evolve in a spatially structured host population then an increase in opportunities for infection of hosts distant to the infected host will lead to selection of parasites with higher virulence (Boots and Sasaki, 1999; van Baalen, 2002). This is because when only local transmission is allowed, parasites cannot afford to be too virulent because they could deplete all local hosts before being transmitted to the next host patch. When global transmission is allowed, this requirement is relaxed. As yet, no experimental tests have been done to test these predictions.

There has been a similar discussion on the relationship between the longevity of parasites in the environment and their optimal virulence level. Ewald has suggested that high longevity of parasites in the environment should select for high virulence, because longer survival in the environment relaxes the parasite need for host and for transmission (Ewald, 1994). Several theoretical studies have attempted to address this question but reached different conclusions. Bonhoeffer et al. (1996) have found that parasite longevity does not affect the optimal level of parasite virulence for endemic infections transmitted exclusively indirectly. Assuming that there is no trade-off between the parasite longevity in the environment and its virulence, the authors found that parasite longevity affects only the R_0 of the infection but not optimal virulence (Bonhoeffer et al., 1996). Gandon (1998) has found that if multiple infections are allowed, higher parasite longevity will select for higher virulence because of the increased strength of the intrahost competition between unrelated parasite strains. Finally, Day (2002c) has found that in cases when there are both direct host-to-host parasite transmission and indirect infection of hosts by parasites in the environment, better parasite survival correlates with higher optimal virulence. The last prediction, however, similar

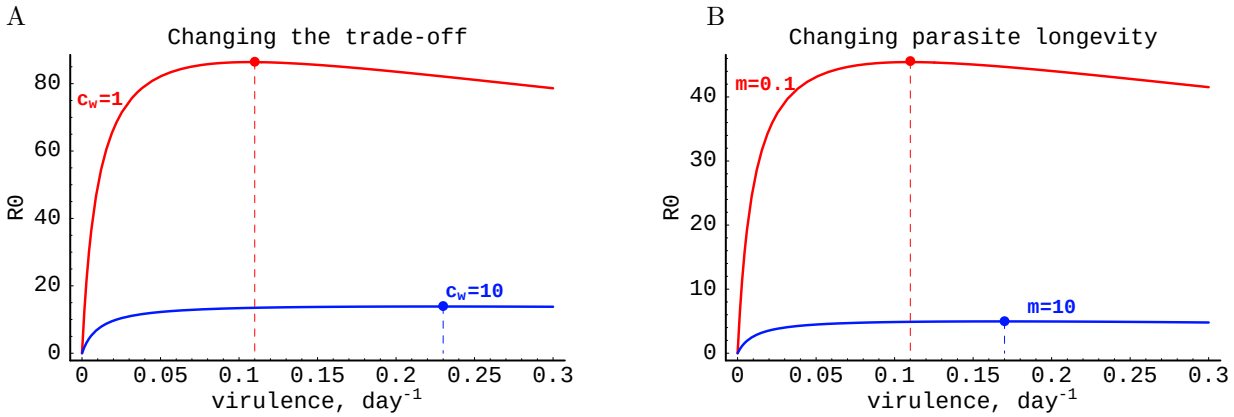

Figure 4: The basic reproductive number of an infection transmitted directly and indirectly as the function of virulence α. The correlation between the transmission rate and virulence is assumed in the form $\beta = \beta_0\alpha/(\alpha^2+c)$ and $\beta_w = \beta_0\alpha/(\alpha+c_w)$, for direct and indirect transmission, respectively. The optimum level of virulence for parasites transmitted only directly $\alpha^*_{direct} \approx 0.18$. The basic reproductive number is calculated in accord with eqn. (5). Panel A shows changes in the optimal level of virulence if the trade-off for indirect transmission is changed: $c_w = 1$ ($\alpha^* \approx 0.11$) and $c_w = 10$ ($\alpha^* \approx 0.23$). Note that depending on the value, parasites transmitted directly and indirectly may evolve higher or lower virulence than parasites transmitted only directly. Panel B shows changes in the optimal level of virulence when the parasite longevity in the environment is changed: $m = 0.1$ ($\alpha^* \approx 0.11$) and $m = 10$ ($\alpha^* \approx 0.17$). Note that in this case, long-lived parasites evolve lower virulence (but the opposite is also possible). If the parasite is transmitted only indirectly, parasite longevity does not affect the optimal level of parasite virulence. Other parameters are: $\beta_0 = 0.01$, $d = 0.001$, $c = c_w = 1$, $\nu = 0.01$, $N = 5\,10^2$, $\rho = 1$, and $m = 0.05$.

to the case with direct and indirect transmission routes, depends heavily on the appropriately chosen trade-offs and needs not be generally true (Figure 4). In summary, these results suggest that it is very difficult to make any general predictions on whether there is any relationship between the route of transmission and optimal virulence unless specific details of the infection (such as trade-offs) are known.

In contrast with parasites, transmitted horizontally, it has been argued that vertically transmitted parasites should be less virulent because in this case transmission of the parasite is linked to the survival of the host (Ewald, 1987). There are several experimental observations that are consistent with this prediction. Bull and coworkers in a series of elegant experiments have shown that increasing opportunities for horizontal transmission of a bacteriophage lead to selection of more virulent viral stains (Bull et al., 1991; Bull and Molineux, 1992; Messenger et al., 1999). Similar results have been obtained for a plasmid evolving in bacteria (Turner et al., 1998). In another experiment, lymphocytic choriomeningitus virus has evolved in a mouse stock from a relatively virulent horizontally transmitted infection to asymptomatic vertically transmitted disease (Traub, 1939). In an exceptional study by Jeon (1972), an initially virulent bacterial parasite of amoebas became harmless after 5 years of strictly vertical transmission where changes in the parasite and the host were responsible for the reduction in virulence. Finally, a comparative study suggests that vertically transmitted lice are less virulent than horizontally transmitted mites while infecting the same host species, rock doves *Columba livia* (Clayton and Tompkins, 1994).

However, there are examples of apparently only vertically transmitted parasites that yet are very virulent. A microsporidian parasite *Tuzetia sp.* of *Daphnia magna* is a strictly vertically transmitted parasite (at least in the laboratory) but yet it dramatically reduces fitness of infected hosts since infected hosts are outcompeted by uninfected hosts (Mangin et al., 1995). Indeed, if some degree of harm is required for transmission, we expect that even vertically transmitted parasites may be virulent. A fundamental vertical transmission equation suggests that virulent parasites of hosts reproducing sexually, may be maintained in the population while being transmitted exclusively vertically. For that the efficacy of vertical transmission from both parents must outweight the reduction in host fecundity and survival (Fine, 1975). Another study suggests that while exclusively vertically transmitted parasites should evolve low virulence, even small rates of horizontal transmission may be sufficient for maintenance of highly virulent parasites that are transmitted vertically with high efficiency (Mangin et al., 1995; Lipsitch et al., 1996).

Finally, parasites experiencing severe bottlenecks during vertical transmission, may evolve lower virulence because of the reduced strength of intra-host competition (Bergstrom et al., 1999). Thus the amount of vertical transmission may indicate of how virulent a parasite is but it needs not be the general rule.

3.2.7 Other factors

There are other factors that may affect the evolution of parasites and which have not been explicitly discussed here. For example, while simple theory assumes that parasites evolve much faster than their hosts, this may not be entirely correct since many hosts species may increase the rate of their evolution by reproducing sexually (Felsenstein, 1988; Ebert and Hamilton, 1996; Ebert, 1999). Clearly in the presence of parasites, hosts will evolve to become more resistant to the infection and this in turn may affect parasite virulence. One good example comes from the coevolution of the myxoma virus and rabbits in Australia, where as hosts evolved high levels of resistance, the virus evolved higher virulence (Fenner and Fantini, 1999, Figure 1). There have been a number of theoretical frameworks attempting to describe how host evolution may occur and what consequences it will have for the parasite evolution (Bowers, 1999, 2001; Gilchrist and Sasaki, 2002; Boots and Bowers, 2003).

Simple theory also assumes that any infected host may in fact infect any susceptible host in the population. While this might be correct for some situations and infections, in general, spatial distribution of hosts may be a critical factor affecting the spread of the infection and consequently the evolution of parasites. Importantly, may predictions of the simple theory cannot be easily extended when there is a spatial heterogeneity in host prevalence. For example, the concept of the basic reproductive number does not work in this case (Haraguchi and Sasaki, 2000; Rauch et al., 2003). Furthermore, parasites may evolve to high or low virulence depending on particular properties of transmission and host connectivity (Boots and Sasaki, 1999; van Baalen, 2002; Read and Keeling, 2003).

Problems with the adaptive theory of parasite evolution

The predictions of the adaptive theory can be only applied to infections for which the trade-offs between parasite characteristics are known to exist. There is no *a priory* knowledge that such trade-offs exist for *any* parasite-host association. Furthermore, changes in parasite virulence need not be always adaptive to the parasite (Bull, 1994; Levin and Bull, 1994), the fact ignored in many interpretations of experimental results.

In previous sections I have reviewed factors that may influence the evolution of parasites even if we assume that there are trade-offs between parasite characteristics. Importantly, the presence of the trade-offs does not determine the exact level of parasite virulence that depends on the precise functions describing the trade-offs (see, for example, Figure 4). In particular, if only the correlation between transmissibility and virulence is known but the trade-off between the host recovery rate and virulence is not, then predictions on how parasites will evolve may be incorrect (Day, 2002b). This can be exemplified by the prediction of the optimal level of the myxoma virus during its evolution in Australia. Based only on the trade-off between the host recovery rate and virus-induced host mortality rate, Anderson and May calculated the optimal level of virus virulence $\alpha^*_{theory} \approx 0.013\ day^{-1}$ that was 3 times lower than the observed value $\alpha^*_{observ} \approx 0.041\ day^{-1}$ (Fenner and Ratcliffe, 1965; Anderson and May, 1982). By adding a trade-off between the rate of transmission and virulence for fleas or mosquitoes, the optimal virulence level became closer to the observed, $\alpha^* \approx 0.040\ day^{-1}$, as shown in Figure 2 (Massad, 1987; Dwyer et al., 1990). Given this uncertainty, it seems that it is very difficult to make any strong predictions on the parasite evolution unless details of the specific host-parasite association are taken into account.

A similar result has been obtained by Ganusov and Antia (2003). The authors found that changes in the rate of parasite transmission or the mechanism of the parasite-induced pathogenesis dramatically alter the optimal level of virulence to which a parasite evolves. Since their approach was based on assumptions of the adaptive theory, this result emphasizes the role of particular details in predicting parasite evolution.

Another limitation of many mathematical models based on the adaptive theory is that the definition of virulence used may not be widely applied to natural infections (Ebert and Bull, 2003). Virulence is often thought as the property of the parasite but host may play a major role in pathogenesis as well (Casadevall and Pirofski, 1999). For example, in infections with noncytopathic viruses pathology may arise because of the immune response destroying virus-infected cells (Krakauer and Nowak, 1999). For some infections, it is not known whether variation in the disease severity is due to infection with different parasite strains or because of the host variability. The adaptive theory, by focusing only on virulence, ignores the evolution of other parasitic traits such as production of toxins or host castration.

Finally, the adaptive theory still does not explain why some parasites are quite avirulent. Can it be that such parasites have evolved to cause little harm in the presence of trade-offs between β, ν and α?

In summary, the adaptive theory of parasite evolution has proven to be a valuable tool in analyzing the evolution of parasites. Yet many limitation of the theory are clear and virulence of many infections is not explained in terms of this theory. Since the field of the evolution of infectious diseases is growing rapidly, future experimental tests will, hopefully, attempt to verify the assumptions behind the adaptive theory as well as test its theoretical predictions for specific infections.

3.3 Non-adaptive hypotheses for the maintenance of parasite virulence

While the hypothesis that virulence can be adaptive is very attractive, it is clear that in many cases parasite virulence is not related to parasite's fitness (transmission) and therefore should be considered as *non-adaptive*. In some cases, this is because such parasites infect hosts that are not normally transmit the parasite to other hosts (Mims et al., 2001). Such accidental infections or "spill-overs" may sometimes be very lethal to the host although many harmless infections most likely occur unnoticed (Ebert, 1998). Infections of this type include soil bacteria *Clostridium tetani* causing tetanus, and bacteria *Clostridium botulinum* causing botulism. Both parasites cause disease in humans by accident, and toxin production by these bacteria most likely has evolved due to other reasons than to kill humans (Lipsitch and Moxon, 1997). Similarly, hantaviruses, Nipah virus, and rabies may cause serious diseases but yet for neither of the infections there is detectable human to human transmission of the parasite (Chua et al., 2000; Kruger et al., 2001; Mims et al., 2001; Lednicky, 2003). It is possible, however, that such spill-overs may with time evolve to begin spreading from human to human without the requirement for the original hosts (Antia et al., 2003). In that case, virulence of such an infection may evolve but how it will evolve would depend on many biological details of the within-host dynamics and epidemiological spread of the parasite.

However, there are some infections, such as *Neisseria meningitidis* and poliovirus, that generally infect many hosts but cause severe disease only in rare occasions (Weiss, 2002). Levin and Bull (1994) have suggested that virulence of such infections may be a result of the short-sighted, within-host parasite evolution. Since natural selection on parasite strains acts not only between infected hosts, but also within the infected host, there might be cases when within-host competition results in emergence of a parasite strain that outcompetes all other strains but leads to the host's death. For example, poliovirus is transmitted through the oral-fecal route and generally does not induce disease. Sometimes, however, it can be passed into the blood and then into the central nervous system (CNS) where it can cause poliomyelitis. Since there is no apparent transmission of the virus from CNS, the authors argued that virulence of poliovirus results from the its within-host evolution (Levin and Bull, 1994). Similar arguments were applied to *N. meningitidis* causing meningitis and HIV causing AIDS.

Since the exact mechanisms by which these parasites cause the disease are not yet understood, such interpretation of parasite virulence has been questioned (Frank, 1996; Ebert, 1999). For example, Ebert (1999) argues that within-host evolution of highly virulent parasite strains may be the direct cost of having high mutation rate required, for example, for evasion of the immune response. Indeed, HIV persists for long periods of time in a given host and during that time it is faced with a constant pressure from the immune system. High mutation rate might be one way of avoiding the recognition by the immune response (Ploegh, 1998). On the other hand, a high mutation rate may have a cost of generating mutants that are able to end the infection by killing the host (Nowak et al., 1991). Similar arguments could be applied to *N. meningitidis* causing a long infection and capable of evolving at a high rate due to phase-shifting (Taha et al., 2002). This alternative explanation generates several specific predictions that could be tested experimentally. An increase in the mutation rate of such parasites should lead, on the one hand, to an increased probability of disease occurrence, and on the other hand, to an increased total transmission from hosts that have not developed the disease. Similarly, decrease of the mutation rate should reduce the total transmission of parasites.

Along the same lines, an alternative explanation for the *N. meningitidis* virulence has been suggested in a recent study by Ancel Mayers et al. (2003). The authors presented a mathematical model where high phase-shifting of the bacteria leads simultaneously to a more rapid epidemiological spread of the infection and a higher probability of causing the disease. In this model, the high mutation rate is advantageous for the parasite because it allows the parasite to adapt faster to a heterogeneous host population to establish the initial asymptomatic infection (Ancel Meyers et al., 2003). In summary, regardless of forces driving evolution of such parasites, within-host evolution may be an important fac-

tor affecting virulence of parasites which, when looked from a between-host viewpoint, may appear to be non-adaptive.

4 Conclusion

In this paper I have reviewed three hypotheses suggested to explain the variation in virulence level between different parasites. In the past 10 years using mathematical models based mainly on the adaptive theory of parasite evolution, many specific predictions of how parasites should evolve in different conditions have been generated. Some of these predictions have been tested and more are hopefully underway. Although currently this research is addressing mostly an academic question, I am optimistic that in the future as more experimental data become available, it may be possible to generate public health recommendations to help "manage" parasite virulence.

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6 Appendix

Optimal virulence level of epidemic and endemic infections

Using a model for the epidemiological spread of directly-transmitted diseases in the absence of intra-host competition, during an endemic parasites evolve to maximize their basic reproductive number, R_0 (Bremermann and Thieme, 1989). At the same assumptions, during an epidemic parasites should evolve to maximize the net rate of the growth in the number of infected hosts, r. Using the definitions for R_0 and r given in eqns. (1)–(2) we obtain the following relationship between two fitness measures:

$$R_0(\alpha) = \frac{r(\alpha)}{\alpha + d + \nu(\alpha)} + 1, \qquad (6)$$

where I assumed that both R_0 and r depend on virulence measured by the parasite-induced host mortality rate α.

I further assume that the trade-offs between the parasite transmissibility, the host recovery rate and virulence are such that the both fitness measures are maximized at intermediate levels of virulence defined in equations:

$$\left.\frac{dR_0(\alpha)}{d\alpha}\right|_{\alpha=\alpha^*_{endem}} = 0, \qquad (7)$$

$$\left.\frac{dr(\alpha)}{d\alpha}\right|_{\alpha=\alpha^*_{epidem}} = 0. \qquad (8)$$

Using eqns. (6) and (7), we obtain

$$\left.\frac{dr(\alpha)}{d\alpha}\right|_{\alpha=\alpha^*_{endem}} = r(\alpha)\left(\frac{1+d\nu/d\alpha}{\alpha+d+\nu}\right)\Bigg|_{\alpha=\alpha^*_{endem}}. \qquad (9)$$

It is then easy to see that if $(1+d\nu/d\alpha)|_{\alpha=\alpha^*_{endem}} > 0$ then $dr/d\alpha|_{\alpha=\alpha^*_{endem}} > 0$. Because $dr/d\alpha|_{\alpha=\alpha^*_{epidem}} = 0$, and $d^2r/d\alpha^2|_{\alpha=\alpha^*_{epidem}} < 0$ (maximum), $\alpha^*_{epidem} > \alpha^*_{endem}$. Thus if the host recovery rate changes slowly with virulence (i.e., $|d\nu/d\alpha| \ll 1$), the optimal virulence level of epidemic infections is always greater than that of endemic infections for any trade-off $\beta = \beta(\alpha)$.

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